

1 Running title: Endometriosis and the risk for miscarriages

2

3 **Endometriosis, especially mild disease: a risk factor for** 4 **miscarriages**

5

6 Alexandra Sabrina Kohl Schwartz M.D.^{a,b}, Monika Martina Wölfler M.D.^{c,d}, Vera Mitter
7 M.Sc.^b, Martina Rauchfuss M.D.^e, Felix Haeberlin M.D.^f, Markus Eberhard M.D.^g,
8 Stephanie von Orelli M.D.^h, Bruno Imthurn M.D.^a, Patrick Imesch M.D.ⁱ, Daniel Fink
9 M.D.ⁱ, Brigitte Leeners M.D.^a

10

11 a. Department of Reproductive Endocrinology, University Hospital Zurich, 8091 Zurich,
12 Switzerland

13 b. Division of Gynecological Endocrinology and Reproductive Medicine, Bern University
14 Hospital, University of Bern, Inselspital, 3010 Bern, Switzerland

15 c. Division of Gynecological Endocrinology and Reproductive Medicine, Medical
16 University of Graz, 8036 Graz, Austria

17 d. Department of Obstetrics and Gynecology, RWTH Aachen University Hospital, 52074
18 Aachen, Germany

19 e. Charité University Hospital Berlin, Department of Psychosomatics, 10117 Berlin,
20 Germany

21 f. Women's Hospital, Cantonal Hospital St. Gallen, 9007 St. Gallen, Switzerland

22 g. Women's Hospital, Cantonal Hospital Schaffhausen, 8208 Schaffhausen, Switzerland

23 h. Stadtspital Triemli, Maternité, 8063 Zurich, Switzerland

24 i. Department of Gynecology, University Hospital Zurich, 8091 Zurich, Switzerland

25

26 Corresponding author:

27 Alexandra Sabrina Kohl Schwartz, M.D.

28 Department of Reproductive Endocrinology,

29 University Hospital Zurich,

30 8091 Zurich, Switzerland

31 alexandra.kohlschwartz@usz.ch

32 alexandra.kohl-schwartz@insel.ch

33 phone: +41 31 632 13 03

34 **Abstract**

35 **Objective:** To investigate the prevalence of miscarriage in women with endometriosis (WwE)
36 compared to disease-free control women (CW).

37 **Design:** Cross-sectional analysis nested in retrospective observational study (n = 940).

38 **Setting:** Women recruited in 9 Swiss, German and Austrian hospitals and associated private
39 practices.

40 **Patient(s):** Previously pregnant women (N= 268) within reproductive age in matched pairs.

41 **Intervention:** Retrospective analysis of surgical reports and self-administered
42 questionnaires.

43 **Main Outcome Measure(s):** Rate of miscarriage, subanalysis for fertility status (≤ 12 vs. > 12
44 months time to conception), endometriosis stages (rASRM I/II vs. III/IV) and phenotypic
45 localisations (superficial peritoneal SUP, ovarian OMA and deep infiltrating DIE
46 endometriosis).

47 **Result(s):** The miscarriage rate is higher in WwE 35.8% (29.6–42.0) compared to CW 22.0%
48 (16.7–27.0); adjusted incidence risk ratio (IRR) of 1.97 (95% CI 1.41–2.75); $p < 0.001$.

49 This remained significant in subfertile WwE 50.0% (40.7–59.4) vs. CW 25.8% (8.5–41.2); $p =$
50 0.017, but not in fertile WwE 24.5% (16.3–31.6) vs. CW 21.5% (15.9–26.8); $p = 0.548$. The
51 miscarriage rate was higher in women with milder forms rASRM I/II 42.1% (32.6–51.4) vs.
52 rASRM III/IV 30.8% (22.6–38.7), compared to 22.0% (16.7–27.0) in CW; $p < 0.001$, and in
53 women with SUP 42.0% (32.0–53.9) compared OMA 28.6% (17.7–38.7) and DIE 33.9%
54 (21.2–46.0) compared to CW 22.0% (16.7–27.0); $p = 0.005$.

55 **Conclusion(s):** Mild endometriosis, as in superficial lesions is related to a great extent of
56 inflammatory disorder, possibly leading to defective folliculogenesis, fertilization and/or
57 implantation presenting as increased risk of miscarriage.

58 **Trial registration number:** NCT 02511626.

59

60 **Key Words:** endometriosis, miscarriage, infertility, pregnancy outcome, superficial peritoneal
61 endometriosis

62 **Introduction**

63 Endometriosis is a chronic and often progressive disease, which is defined by endometriotic
64 tissue outside the uterine cavity that is sensitive to cyclic steroid hormone regulation (1-3).
65 With a prevalence of 6 - 10% of the female population, it is one of the most important benign
66 gynecological diseases in women in their reproductive age (4). Endometriosis is associated
67 with dysmenorrhea, dyspareunia, or chronic pelvic pain. Endometriosis is known to reduce
68 female fertility (4-6) and has an impact on the obstetric outcome of affected women (7-10).
69 In WwE multifactorial reasons result in a reduction in fertility: Reduced tubal motility and
70 passage (11,12), inhibiting inflammatory factors deriving from the peritoneal fluid (13), and a
71 diminished quality of the oocytes affect the chances of a successful implantation after natural
72 conception as well as after assisted reproductive technology (ART) (14-17). However, not all
73 the underlying mechanisms are yet fully understood. Over the last few years, the
74 improvements in pharmacological, surgical, and ART treatment led to an increasing number
75 of pregnancies in WwE (18). The success of pregnancy depends on the placentation and the
76 endometrial function later in pregnancy (19,20). Abnormal local estrogen production and an
77 altered endometrial response to progesterone (progesterone resistance) result in a changed
78 microenvironment. This coats the embryo in early pregnancy (21,22) possibly affecting the
79 "quality of implantation". Miscarriage is the most common complication during the first
80 trimester with an incidence rate of 30 - 50% in the general population, depending primarily on
81 the age of the women (23,24). There is no conclusive answer to the question of whether or
82 not miscarriage rates are increased in WwE: Earlier clinical studies investigated primarily the
83 effect of surgical treatment (ablation of endometriotic lesions) on the prevalence of
84 pregnancy complications; these studies were not adjusted for age (25-27). Some newer
85 studies considering age as a risk factor and showed an association of endometriosis with
86 previous pregnancy losses (8,9,28), but others did not (7, 30-32). A large Canadian cohort
87 study over 12 years with registry data from 784 WwE and 30,284 CW reported a significantly
88 higher rate of miscarriages in WwE (odds ratio 1.89, 95% CI 1.23 – 2.93); however, the
89 diagnostic quality of endometriosis was limited (8). The most recent study on previously
Miscarriages_Manuscript_20170613

90 pregnant women with and without endometriosis was conducted at a specialized referral
91 center. It found a higher miscarriage rate in endometriosis-affected women 29.1% (23.9 –
92 34.3) compared to control women 19.4% (16.1 – 22.7), $p = 0.001$ (7). Because of the high
93 number of women with progressive disease (several surgeries before inclusion in the study)
94 and severe endometriosis lesions, women with mild or asymptomatic disease were rather
95 underrepresented in this collective.

96 Our primary aim was to evaluate and compare miscarriage rates in a population of women
97 with surgically confirmed endometriosis and a broad variety of clinical manifestations; in
98 order to reflect the average female population affected by endometriosis. To improve our
99 understanding of the association between endometriosis and miscarriage, we included in the
100 analysis the phenotypical disease localization and the fertility status.

101

102 **Methods**

103 This is a cross-sectional analysis about the prevalence of miscarriages nested in a
104 retrospective observational study ($n = 940$) on the quality of life in WwE.

105

106 Questionnaire

107 We designed a questionnaire focusing on the women's health and obstetric history. We
108 collected sociodemographic data and asked the women whether or not they had difficulties
109 conceiving and for how long they tried to become pregnant.

110

111 Recruitment

112 Women from the ages of 18 to 45 were recruited between December 2010 and December
113 2015 at participating hospitals or associated private practices. Participants were recruited at
114 university hospitals in Switzerland, Germany and Austria (University Hospital Zurich, Charité
115 – Medical University Berlin, University Hospital of Graz, and RWTH Aachen University),
116 cantonal hospitals in Switzerland (Schaffhausen, Winterthur, St. Gallen, Solothurn), the
117 Stadtsptial Triemli Zurich and in associated private medical practices. Inclusion criteria were

118 defined as not being pregnant and being able to complete the questionnaire (without
119 linguistic, mental or psychological impairment). Patients having undergone surgery for the
120 diagnosis of endometriosis and fulfilling inclusion criteria were invited to participate and
121 asked for informed consent. CW without clinical suspicion of endometriosis (no severe
122 dysmenorrhoea, no heavy cyclic abdominopelvic pain) fulfilling the inclusion criteria were
123 recruited during annual check-ups. In total 647 women affected by endometriosis and 666
124 CW were invited to participate. Of those invited, written consent was received from 505 WwE
125 (78.0%), and 435 CW (65.3%) respectively.

126

127 Surgical reports

128 Surgical reports for each surgical intervention were obtained from the patients' clinics.
129 From 468 out of 505 WwE (92.7%), we obtained surgical reports with sufficient details to do
130 a correct staging and grading of the lesions. In 438 of 468 WwE (93.6%), the diagnosis was
131 confirmed histologically after surgical resection; 30 of 468 WwE (6.4%) were diagnosed
132 surgically, primarily after laser evaporation of light lesions. To avoid bias, all surgical and
133 histological records were reviewed by two blinded investigators.

134 The documents reviewed contained the total number of endometriosis-associated surgeries,
135 the histological confirmation of endometriosis, a description of the localization and size of
136 endometriosis lesions and the dimensions of adhesions. The revised classification of the
137 American Society for Reproductive Medicine (rASRM) (33) was used to categorize the
138 endometriosis into the four stages. This staging was applied, as it was initially developed to
139 define chances for pregnancy (34). Additionally, the lesions were classified into three
140 phenotypes: superficial peritoneal endometriosis (SUP), ovarian endometriosis (OMA), and
141 deep infiltrating endometriosis (DIE), as previously described by other investigators (35,36).
142 DIE lesions were classified as ≥ 5 mm deep invasive endometriotic lesions. (2,37). As those
143 three different phenotypes often present together, patients were classified according to their
144 most severe lesion, used for grading as follows: DIE >OMA >SUP (35,38).

145

146 Statistical analysis

147 We analyzed responses regarding the number of previous miscarriages, the number of
148 pregnancies conducted beyond the 24th gestational week, and the number of deliveries.
149 All data were stored in a computerized database. Statistical analysis was performed using
150 STATA statistical software Version 14 (Stata Corporation, College Station, TX, USA). Data
151 were presented as absolute numbers (n) and as percentages, with 95% confidence intervals
152 (95% CIs). Differences among groups were analyzed by chi-square tests for categorical data;
153 the Student's t-test was used for quantitative data. Miscarriage rates were defined as the
154 rate of previous failures relative to the total number of previous pregnancies. We calculated
155 the miscarriage rate for the rASRM stages and the most severe phenotypic endometriosis
156 localization in fertile and subfertile subpopulations. Adjustments were made for known risk
157 variables: age (≤ 29 , 30-35, ≥ 35 years), BMI (≤ 19 , 20-24, 25-29 and ≥ 30 kg/m²) and smoking
158 (never a smoker, previous smoker, and current smoker) as categorical variables. Due to
159 missing values (18/268), multiple imputations were made for "smoking". "Previous
160 miscarriage" is not independent in our population, because women can have more than one
161 miscarriage. Therefore, we applied a hierarchical mixed effect model (Poisson regression) to
162 account for the matched pairs (level I) and for pregnancies within the same woman (level II).
163 We calculated the rate of miscarriages/pregnancies within our population (39). The model
164 was chosen because each event has a defined onset (pregnancy) with a defined endpoint
165 (miscarriage or delivery). The level of significance was set at $p < 0.05$ and $p < 0.001$ for
166 multiple testing.

167

168 Ethics

169 The regional ethical review committees as well as the ethic boards of the participating
170 hospitals in Switzerland, Germany, and Austria approved the study. All women provided
171 signed informed consent for study participation as well as verification of endometriosis
172 diagnosis through their medical charts. The trial was conducted in accordance with the

173 Declaration of Helsinki. It was registered at clinicaltrials.gov, under the reference number
174 NCT 02511626.

175

176 **Results**

177 Study population

178 For this analysis, woman with previous pregnancies were selected as shown in Figure I. In
179 the final analysis we included 143 WwE matched for age with 143 CW, out of 203 (total n =
180 286). Most WwE were born within 12 months of their matching CW (120 pairs) the remaining
181 were born at most 24 months apart (23 pairs). Of the 286 women, a total of 508 previous
182 pregnancies was analyzed.

183 Patient characteristics of 143 WwE and 143 CW were analyzed (Table 1). There were no
184 significant differences in the characteristics of the two groups beside the lower parity in WwE:
185 a total number of 152 children were delivered by WwE, compared to 204 children born of
186 CW; 32 WwE (22.4%) and 13 CW (9.1%) were nulliparous; $p = 0.001$.

187 Among WwE, 33 were staged with rASRM I (23.1%), 26 with rASRM II (18.2%), 42 with
188 rASRM III (29.4%) and 42 with rASRM IV (29.4%). The phenotypically most severe
189 endometriosis lesion was a SUP in 48 women (33.6%), an OMA in 47 women (32.8%), and a
190 DIE in 48 women (33.6%). Of the 48 women with DIE, 33 had cul de sac lesions, 11 had an
191 invasive lesion on the sacrouterine ligament and 4 had invasive peritoneal lesions of other
192 localisations. The mean number of surgical interventions related to endometriosis per WwE
193 was 1.87 (range 1-9).

194

195 Miscarriages

196 In our analysis, 240 pregnancies in WwE and 268 pregnancies in CW were included (Figure
197 I). The total number of miscarriages per woman was similar in both groups: 36 WwE and 30
198 CW had one previous miscarriage; 13 WwE and eight CW had two previous miscarriages.
199 Recurrent miscarriages, with three or more miscarriages (according to the ESHRE

200 classification, 40), occurred in a total of 11/286 (3.9%) women, 7/143 (4.9%) WwE, and
201 4/143 (2.8%) CW, $p = 0.874$.

202 Table 2 shows miscarriage rates in relation to fertility, localization of the most severe lesions,
203 and disease stage. Generally, the miscarriage rate was significantly higher in WwE
204 compared to CW (Table 2). This difference remained significant for subfertile women
205 independent of previous ART, but the miscarriage rate for fertile WwE did not differ from the
206 miscarriage rate for fertile CW.

207 Mild and severe endometriosis were both associated with a higher prevalence of
208 miscarriages compared to CW. This relationship was stronger in mild endometriosis (rASRM
209 I/II) than in severe endometriosis (rASRM III/IV), compared to CW. The miscarriage rate was
210 highest in women diagnosed with SUP, followed by women diagnosed with DIE, and then by
211 women diagnosed with OMA.

212 Our regression model confirmed a higher rate of previous miscarriages for WwE compared to
213 CW (Table 3), also after adjustment for risk factors for miscarriage such as age, BMI,
214 subfertility, and smoking habit. The adjusted IRR for previous miscarriages was significantly
215 increased in subfertile but not in fertile WwE. The adjusted IRR for previous miscarriages
216 was significantly higher in cases of mild and of superficial peritoneal endometriosis compared
217 to CW.

218

219 **Discussion**

220 This study shows a higher rate of previous miscarriages in WwE, especially in women with
221 minimal or mild disease, compared to CW in the adjusted analysis. We show that primarily
222 women with milder forms of endometriosis as well as subfertile woman are affected by
223 miscarriages. This allows a clearer picture among different types of endometriosis and
224 indicates that the diagnosis of endometriosis should not generally be associated with a
225 higher risk for miscarriage. It also means that young women with early stages of
226 endometriosis, a good ovarian reserve and tube motility should be reassured and
227 encouraged to try for a spontaneous conception.

228

229 A strength of this study is the inclusion of women recruited in secondary, and tertiary centers
230 as well as in private practices: this distribution offers a considerably representative sample of
231 the female population treated for endometriosis. While many women diagnosed with
232 endometriosis suffer from severe pain symptoms and a decreased quality of life, others
233 experience fewer pain symptoms and a quality of life similar to that of the general population
234 (41). WwE treated in secondary and tertiary centers seem to differ in disease symptoms and
235 associated quality of life (42) from women treated in primary care centers. As most studies
236 focus on the presence of disease symptoms, WwE with few symptoms and satisfactory
237 quality of life are underrepresented in most scientific studies conducted at university care
238 centers. Our recruitment strategy was applied to overcome this selection bias and reflect the
239 average female population affected and treated by endometriosis.

240

241 Because of the heterogeneity of endometriosis lesions, classification systems often show
242 only weak associations with disease symptoms (41). Another strength of this study, is the
243 application of both the well-known classification system of the American Society for
244 Reproductive Medicine (rASRM; 33) and the surgical phenotypic classification of
245 endometriosis adopting the description of the most severe endometriosis lesion (SUP <OMA,
246 <DIE) (36). It has been shown that phenotypic classification corresponds better to the
247 obstetric outcome than the rASRM classification (42,43). The even distribution of SUP, OMA,
248 and DIE as the most severe localization of the endometriosis disease within our study
249 population allows for a reliable evaluation of the role of different lesion locations in the risk for
250 miscarriage.

251 Furthermore the analysis based on the mixed-effects Poisson regression model accounting
252 for the non-independence of pregnancies within the same women delivers very reliable
253 results. Recall bias was minimized through the selection of women with previous
254 pregnancies for evaluation within an observational study on quality of life in WwE, e.g. the
255 women were not aware of this particular investigation's hypothesis. The sample size was

256 large and the age ranges particularly narrow: matching in the present study provided a
257 control sample out of the same population. This is particularly important because age is the
258 most important risk factor for miscarriages.

259 The following limitations have to be taken into consideration: data analysis was based on
260 questionnaire information; beside reporting on endometriosis related surgery, no other
261 medical records were reviewed, diagnosis of endometriosis was based on surgical and
262 histological reports. Laparoscopy with or without histological verification is widely used to
263 diagnose and rule out the presence of endometriosis. However, the correct diagnosis of
264 endometriosis depends highly on the abilities of the surgeon performing the laparoscopy.
265 Hidden endometriosis lesions retroperitoneally or vaginally can be easily missed, especially if
266 the patient has not been thoroughly examined preoperatively (44) In the past surgical
267 judgment was proven to be equivalent to histological diagnosis (45). Surgical and histological
268 reports did not allow inclusion of possible adenomyotic lesions, because correct diagnosis
269 would need specific ultrasound assessment of the uterine morphology (46). Uterine
270 adenomyosis is known to frequently coexist with endometriosis (15) and is associated with
271 an increased risk for miscarriages (47).

272 Acquired information about infertility and reasons for subfertility and ART treatment is based
273 on the patients' answers. Pregnancy, the loss of a pregnancy and deliveries are life events,
274 so recall is most likely correct and, if biased, then for WwE and CW in the same manner. In
275 women affected with endometriosis, sensitivity to possible infertility might be higher, as it is
276 known and often communicated, that endometriosis might affect fertility. This could lead to
277 an information bias and could affect the detection of a pregnancy different from that of CW.

278 As a further limitation, asymptomatic endometriosis in the control group cannot be excluded,
279 since not all of the CW had surgical treatment and were included into the study after the
280 exclusion of symptoms suspicious for endometriosis. This recruitment was the best possible,
281 since recruitment of women undergoing laparoscopy for gynecological non-endometriosis
282 reasons such as e.g. tubal ligation or removal of benign ovarian cysts have an affected
283 fertility, so they impose a selection bias. However, missed diagnosis of endometriosis in the

284 control group would lead to an overestimation of the described effect. The recruitment in
285 different centres reduces selection bias in regard to similarity with the general population,
286 both in WwE and CW. Because of the number of missing answers for thyroid disease, and
287 immunological disorders, we could not include these specific risk factors into our mixed-effect
288 regression model.

289

290 Our results are in agreement with large registry-based studies (8,48), showing a higher
291 prevalence of miscarriages in women diagnosed with endometriosis than in CW. Studies
292 showing no difference in miscarriage rates between women with and without endometriosis
293 focus either on ART-treated women (31,32) or women with surgically treated septate uteri
294 (30). Both of these conditions might imply a higher impact on miscarriage rates than the
295 endometriosis itself. The following mechanisms support our results indicating an association
296 between endometriosis and miscarriages: pathophysiologically, an impaired folliculogenesis
297 and an insufficient endometrial function in WwE have been postulated. The eutopic
298 endometrium may not function normally in WwE: inflammation processes lead to an
299 increased release of reactive oxygen species (ROS) and an increased expression of
300 enzymes (17). These enzymes induce an accumulation of free radicals in cells near and in
301 the endometrium, which possibly affect implantation (17). Additionally, ROS affect the mitotic
302 spindle as well as the separation of chromosomes and consequently delay completion of
303 meiosis I during fertilization (49,50); this could provide an explanation for a lower oocyte
304 quality in endometriosis, as shown in animal studies (51).

305 Physiologically, progesterone induces the decidualization of stromal cells of the endometrium
306 to become receptive for the embryo (52). As a consequence of progesterone resistance in
307 endometriosis, this process may become dysregulated and lead to suboptimal implantation
308 (6). Endometriosis affected tissue shows different pathological features, for example delayed
309 maturation, altered glycosylation and molecular abnormalities such as alterations in local
310 steroid biosynthesis, cell growth, apoptosis, immune-cell function, angiogenesis, cell
311 adhesion, and cytokine production, all of which might reduce chances of a successful

312 pregnancy outcome (14, 53, 54, 55). In the literature the use of anti-inflammatory hormonal
313 (56,57) and non-hormonal (58) treatments for pain symptoms have been examined. It is
314 recommended that they be investigated further for their potential to improve implantation and
315 reduce miscarriages.

316 In addition, in endometriosis inadequate uterine contractions occur throughout the menstrual
317 cycle; the frequency, amplitude and basal pressure have been shown to be higher and
318 possibly favor miscarriages (17).

319

320 The higher miscarriage rate in lower stages of endometriosis detected in our study is in line
321 with findings from other authors: the recent analysis by Santulli *et al.* (9) showed a higher
322 rate of miscarriages for all phenotypes of endometriosis e.g. SUP, OMA and, DIE compared
323 to CW. As in our study, the highest miscarriage rate was reported for “mildest” SUP lesions
324 (n = 33/87; 37.9%) vs. OMA lesions (n = 28/104; 26.9%) vs. DIE lesions (n = 78/287; 27.2%).

325 In both studies, the higher rate of previous miscarriages in women with SUP could be the
326 result of a higher pregnancy rate among these women: women with more severe lesions
327 such as OMA or DIE are more likely to have a lower reproductive performance (59,60) or
328 poor motility of the fallopian tubes (11), with consecutively lower spontaneous over all
329 pregnancy rates.

330 In contrast, a meta-analysis of reproductive outcomes by Barbosa (60) did not show a
331 significant difference in miscarriages between mild and severe endometriosis-affected
332 women. Only 21 out of 92 studies included in this meta-analysis reported rASRM staging of
333 endometriosis, so evidence in relation to ASRM staging was considered low.

334 Pathophysiological mechanisms might explain the finding of increased miscarriage rates in
335 WwE but also especially in women with mild endometriosis: early stages of the disease with
336 more active lesions are known to lead to a more inflammatory milieu (61,62), compared to
337 the more scarring lesions of higher disease stages (63). Fresh endometriosis lesions seem to
338 be associated with an inflammatory response represented by overproduction of
339 prostaglandins, metalloproteinases, cytokines, and chemokines (3). The resulting

340 inflammatory process impairs ovarian, peritoneal, tubal, and endometrial function, and may
341 lead to defective folliculogenesis by altered follicular milieu because of increased rates of
342 granulosa cell apoptosis (64) as well as increased concentrations of follicular fluid natural
343 killer cells and lymphocytes (65), fertilization, and/or implantation (5). However it has been
344 shown very recently, that the rate of aneuploidy rate in patients affected by endometriosis is
345 not increased (66). This could lead us to the speculation that the impact of mild
346 endometriosis on fertility might be due to non-genetic rather metabolic intracellular
347 processes; so either insufficient oocyte maturation with retardation of the embryo before
348 reaching blastocyst stage or on insufficient embryonal development either because of altered
349 endometrial receptivity (67) and therefore reduced „supply“ or an impairment of intracellular
350 metabolism probably also in trophoblast development.

351 There is an association of miscarriages in subfertile women with and without endometriosis.
352 Additionally, subfertile women often require ART, an intervention possibly associated with
353 complications later in pregnancy such as e.g. preterm birth and low birth weight (68). ART
354 itself does not pose a risk for miscarriages, but the underlying specific reproductive disorders
355 and the increased age, both affecting oocyte quality are of very high importance when
356 assessing miscarriage risk (69) in these women.

357 Based on this knowledge, our results of highest IRR for subfertile women with endometriosis
358 are not surprising. In contrast our finding that there is no difference in the prevalence of
359 miscarriages between fertile WwE and fertile CW is very important for counseling.

360 Our findings are in contrast to the above-mentioned recent study (9), where the risk for
361 miscarriages in fertile women with endometriosis (67/341; 19.6%) was significantly higher
362 than in control women (72/583; 12.3%). However, the miscarriage rates in both of these
363 groups were lower compared to our and other studies (70). The reason for the differences in
364 these results might be the older age of women, e.g. the higher risk for miscarriage at the time
365 of inclusion into our study.

366 In conclusion, our study found that miscarriage rates are higher in endometriosis-affected
367 women, especially in women with mild lesions and those suffering from infertility. Further

368 studies, especially prospective controlled studies, are necessary to confirm our findings. A
369 retrospective study such as this, with the attendant limitations of study design, cannot
370 definitely prove the hypothesis. Analyzing underlying pathophysiological mechanisms such
371 as the folliculogenesis and the endometrial function may help to better understand the
372 etiology of miscarriages and develop effective treatments to improve chances for successful
373 pregnancy in women with endometriosis.

374

375 Acknowledgements

376 The authors thank Kathryn Imboden for editing of the manuscript, Lukas Buetikofer for
377 statistical consulting and to Brigitte Alvera, Anna Dietlicher, Franka Grischott, Nicole
378 Kuenzle, Judith Kurmann, Christina Liebermann, Karoline Stojanov, Elvira Gross, Lina
379 Looser, Sarah Schaerer, Theodosia Charpidou, Elena Lupi and Franziska Graf for their
380 assistance in data collection.

381

382 **Figure legends**

383 Figure 1

384 Patient inclusion flow-chart

385 WwE: Women with endometriosis, CW: Control women

386 Table 1

387 Patient characteristics

388 WwE: Women with endometriosis, CW: Control women

389 Data reported as mean \pm standard deviation or number (%)

390 a: chi-square test; b: t-test

391 Table 2

392 N= number of miscarriages/ pregnancies

393 WwE: Women with endometriosis, CW: Control women

394 a: chi-square test

395 Rates of previous miscarriages according infertility and rates of previous miscarriages according
396 surgery

397 rASRM: revised American Society of Reproductive Medicine classification (ASRM 1997)
 398 Surgical phenotypical classification (Chapron et al. 2010)
 399 SUP: superficial endometriosis; OMA: ovarian endometrioma; DIE: deep infiltrating endometriosis
 400 Table 3
 401 Incidence rate ratio (IRR) of previous miscarriages from the mixed-effects Poisson regression analysis
 402 WwE: Women with endometriosis, CW: Control women
 403 a: adjusted for age, BMI, smoking* habits and subfertility
 404 *: imputation for smoking because of missing answers
 405 rASRM: revised American Society of Reproductive Medicine classification (ASRM 1997)
 406 Surgical phenotypical classification (Chapron et al. 2010)
 407 SUP: superficial endometriosis; OMA: ovarian endometrioma; DIE: deep infiltrating endometriosis

408
409
410
411
412
413
414
415
416
417
418
419
420
421
422

Table 1	Women with endometriosis Control women		p- value
	(WwE)	(CW)	
	N = 143	N = 143	
Age (years)	37.34 ± 4.83	37.45 ± 4.78	0.898 ^a
BMI (kg/m ²)	23.10 ± 4.24	23.47 ± 3.77	0.584 ^a
Smoking habits (n, %)			
Never smoked	66 (46.1%)	77 (53.8%)	0.301 ^a
Previous smoker	46 (32.2%)	37 (25.9%)	
Current smoker	23 (16.1%)	18 (12.6%)	

433	Not available	8 (5.6%)	11 (7.7%)	
434				
435	Gravidity (n, %)			
436	1	83 (58%)	64 (44.7%)	0.071^a
437	2	37 (29.5%)	52 (36.4%)	
438	≥3	23 (16.1%)	27 (23.9%)	
439	Parity (n, %)			
440	0	32 (22.4%)	13 (9.1%)	0.001^a
441	1	75 (52.4%)	67 (46.8%)	
442	2	31 (21.7%)	52 (36.4%)	
443	≥ 3	5 (3.5%)	11 (7.7%)	
444				
445	Immunological disease (n,%)	14 (9.8%)	7 (4.9%)	0.890 ^b
446	Thyroid disease (n, %)	22 (15.4%)	19 (13.3%)	0.377 ^b
447				
448				
449				
450				

Table 2

	Endometriosis women (WwE) (total N= 240)			Control women (CW) (total N= 268)			
	N	Rate % (95% CI)		N	Rate % (95% CI)		p- val ^a
452							
453							
454	Total	86/240	35.8 (29.6 – 42.0)	59/268	22.0 (16.7 – 27.0)		<0.001
455	Fertile women	32/132	24.5 (16.3 – 31.6)	51/237	21.5 (15.9 – 26.8)		0.548
456	Subfertile women	54/108	50.0 (40.7 – 59.4)	8/31	25.8 (8.5 - 41.2)		0.017
457	Previous ART	11/20	55.0 (36.5 – 76.8)	1/19	5.3 (0.1 – 15.3)		<0.001

	SUP			OMA			DIE			Controls			
	N	Rate % (95% CI)		N	Rate % (95% CI)		N	Rate % (95% CI)		N	Rate % (95% CI)		p- val ^a
458													
459													
460													
461													
462	34/79	43.0 (32.0 – 53.9)		22/77	28.6 (17.7 – 38.7)		20/59	33.9 (21.2 – 46.0)		59/268	22.0 (16.7 – 27.0)		0.005
463	rASRM I/II			rASRM III/IV			Controls						
464													
465	45/107	42.1 (32.6 – 51.4)		41/133	30.8 (22.6 – 38.7)					59/268	22.0 (16.7 – 27.0)		<0.001

Table 3

	Unadjusted IRR (95% CI)	p-value	Adjusted ^a IRR (95% CI)	p-value
466				
467				
468				
469				
470	Ref		Ref	
471	1.62 (1.17 – 2.27)	0.004	1.97 (1.41 – 2.75)	<0.001
472				
473	Ref		Ref	
474	1.94 (0.92 – 4.07)	0.081	2.41 (1.01 – 5.78)	0.048
475				

476	Fertile CW	Ref		Ref	
477	Fertile WwE	1.13 (0.72 – 1.75)	0.597	1.11 (0.7 – 7.76)	0.657
478					
479					
480	CW	Ref		Ref	
481	WwE with rASRM lesion I/II	1.91 (1.29 – 2.82)	0.001	1.57 (1.00 - 2.44)	0.046
482	WwE with rASRM lesion III/IV	1.40 (0.94 – 2.08)	0.098	1.19 (0.76 - 1.85)	0.446
483	WwE with SUP lesion	1.98 (1.33 – 2.95)	0.001	1.67 (1.07 - 2.61)	0.024
484	WwE with OMA lesion	1.32 (0.81 – 2.14)	0.256	1.11 (0.65 - 1.88)	0.703
485	WwE with DIE lesion	1.49 (0.91 – 2.43)	0.110	1.19 (0.70 - 2.61)	0.515

486

487 References corrected:

488

- 489 1. [Practice Committee of the American Society for Reproductive Medicine.](#)
490 Endometriosis and infertility. Fertil Steril 2006; 86:S156-60.
- 491 2. The international glossary on infertility and fertility care, 2017. Fertil Steril.
492 2017, in press
- 493 3. Bulun SE, Endometriosis. New Engl J Med 2009;360:268–79.
- 494 4. Giudice LC, Clinical practice. Endometriosis. New Engl J Med
495 2010;362:2389-98.
- 496 5. Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A.
497 Pathogenic mechanisms in endometriosis-associated infertility. Fertil Steril
498 2008;90:247-57.
- 499 6. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis:
500 pathogenesis and treatment. Nat Rev Endocrinol 2014;10:261–75.
- 501 7. Vercellini P, Parazzini F, Pietropaolo G, Cipriani S, Frattaruolo MP, Fedele
502 L. Pregnancy outcome in women with peritoneal, ovarian and rectovaginal
503 endometriosis: a retrospective cohort study. BJOG 2012;119:1538–43.
- 504 8. Aris, A. A 12-year cohort study on adverse pregnancy outcomes in Eastern
505 Townships of Canada: impact of endometriosis. Gynecol Endocrinol 2014;
506 30:34–7.
- 507 9. Santulli P, Marcellin L, Menard S, Thubert T, Khoshnood B, Gayet V, et al.
508 Increased rate of spontaneous miscarriages in endometriosis-affected
509 women. Hum Reprod 2016;31:1014-23.
- 510 10. Leone Roberti Maggiore U, Ferrero S, Mangili G, Bergamini A, Inversetti A,
511 Giorgione V, et al. A systematic review on endometriosis during pregnancy:
512 diagnosis, misdiagnosis, complications and outcomes, Hum Reprod Update
513 2016;22:70–103.
- 514 11. Almeida, O. D. Microlaparoscopy and a GnRH agonist: a combined
515 minimally invasive approach for the diagnosis and treatment of occlusive
516 salpingitis isthmica nodosa associated with endometriosis. JSLS
517 2005;9:431–3.
- 518 12. Fortier KJ, Haney AF. The pathologic spectrum of uterotubal junction
519 obstruction. Obstet Gynecol 1985;65:93–8.
- 520 13. Lyons RA, Djahanbakhch O, Saridogan E, Naftalin AA, Mahmood T,
521 Weekes A, et al. Peritoneal fluid, endometriosis, and ciliary beat frequency in
522 the human fallopian tube. Lancet 2002;360:1221–22.
- 523 14. Carvalho L, Podgaec S, Bellodi-Privato M, Falcone T, Abrão MS. Role of
524 eutopic endometrium in pelvic endometriosis J Minim Invasive Gynecol
525 2011;18:419-27.

- 526 15. Brosens I, Brosens JJ, Fusi L, Al-Sabbagh M, Kuroda K, Benagiano G.
527 Risks of adverse pregnancy outcome in endometriosis. *Fertil Steril*
528 2012;98:30-5.
- 529 16. Hanzman EE, Garcia-Velasco JA, Pellicer A. Oocyte donation and
530 endometriosis: What are the lessons? *Semin Reprod Med* 2013;31:173–7.
- 531 17. Benagiano G, Brosens I, Habiba M. Structural and molecular features of the
532 endomyometrium in endometriosis and adenomyosis. *Hum Reprod Update*.
533 2014;20:386-402.
- 534 18. de Ziegler D, Borghese, B., Chapron, C. Endometriosis and infertility:
535 pathophysiology and management. *Lancet* 2010;376:730–38.
- 536 19. Brosens I, Pijnenborg R, Vercruyse L, Romero R. The “Great Obstetrical
537 Syndromes” are associated with disorders of deep placentation. *Am J*
538 *Obstet Gynecol* 2011;204:193–201.
- 539 20. Marcellin L, Santulli P, Gogusev J, Lesaffre C, Jacques S, Chapron C, et al.
540 Endometriosis also affects the decidua in contact with the fetal membranes
541 during pregnancy. *Hum Reprod* 2015;30:392-405.
- 542 21. Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, et al.
543 Progesterone resistance in endometriosis: link to failure to metabolize
544 estradiol. *Mol Cell Endocrinol* 2006;248:94–103.
- 545 22. Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, et al.
546 Gene expression analysis of endometrium reveals progesterone resistance
547 and candidate susceptibility genes in women with endometriosis.
548 *Endocrinology* 2007;148:3814–26.
- 549 23. Knudsen UB, Hansen V, Juul S, Secher NJ. Prognosis of a new pregnancy
550 following previous spontaneous abortions. *Eur J Obstet Gynecol Reprod*
551 *Biol.* 1991 39:31-6.
- 552 24. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal
553 age and fetal loss: population based register linkage study. *BMJ* 2000;
554 320:1708-12.
- 555 25. Naples JD, Batt RE, Sadigh H. Spontaneous abortion rate in patients with
556 endometriosis. *Obstet Gynecol* 1981;57:509-12.
- 557 26. Wheeler JM, Johnston BM, Malinak LR. The relationship of endometriosis to
558 spontaneous abortion *Fertil Steril* 1983;39:656-60.
- 559 27. Groll, M. Endometriosis and spontaneous abortion. *Fertil Steril*
560 1984;41:933–35
- 561 28. Metzger DA, Olive DL, Stohs GF, Franklin RR. Association of endometriosis
562 and spontaneous abortion: effect of control group selection. *Fertil Steril*
563 1986;45:18-22
- 564 29. Omland AK, Abyholm T, Fedorcsák P, Ertzeid G, Oldereid NB, Bjercke S, et
565 al. Pregnancy outcome after IVF and ICSI in unexplained, endometriosis-
566 associated and tubal factor infertility. *Hum Reprod* 2005;20:722-27.
- 567 30. Gergolet M, Gianarloi L, Kenda Suster N, Verdenik I, Magli MC, Gordts S.
568 Possible role of endometriosis in the aetiology of spontaneous miscarriage
569 in patients with septate uterus. *Reprod Biomed Online* 2010;21:581-85.
- 570 31. Mekar K, Masamoto H, Sugiyama H, Asato K, Heshiki C, Kinjyo Tet al.
571 Endometriosis and pregnancy outcome: are pregnancies complicated by
572 endometriosis a high-risk group? *Eur J Obstet Gynecol Reprod Biol*
573 2014;172:36-9
- 574 32. Leonardi M, Papaleo E, Reschini M, Pagliardini L, Benaglia L, Candotti G, et
575 al. Risk of miscarriage in women with endometriosis: insights from in vitro

- 576 fertilization cycles. *Fertil Steril* 2016;106:386-92.
- 577 33. Schenken, R. S., Guzick, D. S. Revised endometriosis classification: 1996.
- 578 *Fertil Steril* 1997;67:815–16.
- 579 34. Rock, J. A., Guzick, D. S., Sengos, C., Schweditsch, M., Sapp, K. C., Jones,
- 580 H. W. The conservative surgical treatment of endometriosis: evaluation of
- 581 pregnancy success with respect to the extent of disease as categorized
- 582 using contemporary classification systems. *Fertil Steril* 1981;35:131–7.
- 583 35. Chapron C, Bourret A, Chopin N, Dousset B, Leconte M, Amsellem-
- 584 Ouazana D, et al. Surgery for bladder endometriosis: long-term results and
- 585 concomitant management of associated posterior deep lesions. *Hum*
- 586 *Reprod* 2010;25:884-9
- 587 36. Somigliana E, Vercellini P, Gattei U, Chopin N, Chiodo I, Chapron C.
- 588 Bladder endometriosis: getting closer and closer to the unifying metastatic
- 589 hypothesis. *Fertil Steril* 2007;87:1287-90.
- 590 37. Koninckx [PR](#), [Martin DC](#). Deep endometriosis: a consequence of infiltration
- 591 or retraction or possibly adenomyosis externa? *Fertil Steril* 1992;58:924-8
- 592 38. Sibiude J, Santulli P, Marcellin L, Borghese B, Dousset B, Chapron C.
- 593 Association of history of surgery for endometriosis with severity of deeply
- 594 infiltrating endometriosis. *Obstet Gynecol* 2014;124:709-17.
- 595 39. Raudenbush SW, Bryk AS. Hierarchical Linear Models: Applications and
- 596 Data Analysis Methods. 2nd edn, 2002. Sage Publications, Thousand Oaks,
- 597 CA.
- 598 40. Kolte AM, Bernardi LA, Christiansen OB, Quenby S, Farquharson RG,
- 599 Goddijn M, et al. ESHRE Special Interest Group, Early Pregnancy.
- 600 Terminology for pregnancy loss prior to viability: a consensus statement
- 601 from the ESHRE early pregnancy special interest group. *Hum Reprod*
- 602 2015;30:495-8.
- 603 41. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG.
- 604 Association between endometriosis stage, lesion type, patient
- 605 characteristics and severity of pelvic pain symptoms: a multivariate analysis
- 606 of over 1000 patients. *Hum Reprod* 2007;22:266–71.
- 607 42. De Graaff AA, Dirksen CD, Simoens S, De Bie B, Hummelshoj L, D'Hooghe
- 608 TM, et al. Quality of life outcomes in women with endometriosis are highly
- 609 influenced by recruitment strategies. *Hum Reprod* 2015;30:1331–41.
- 610 43. Vignano P, Corti L, Berlanda N. Beyond infertility: obstetrical and postpartum
- 611 complications associated with endometriosis and adenomyosis. *Fertil Steril*
- 612 2015;104:802-12.
- 613 44. Wykes CB, Clark TJ and Khan KS. Accuracy of laparoscopy in the
- 614 diagnosis of endometriosis: a systematic quantitative review. *BJOG* 2004;
- 615 111:1204–1212.
- 616 45. Fauconnier, A., Chapron, C., Dubuisson, J.-B., Vieira, M., Dousset, B.,
- 617 Bréart, G. Relation between pain symptoms and the anatomic location of
- 618 deep infiltrating endometriosis. *Fertil Steril* 2002;78:719–26.
- 619 46. Guerriero S, Condous G, Van den Bosch T, Valentin L, Leone FP, Van
- 620 Schoubroeck D, et al. Systematic approach to sonographic evaluation of the
- 621 pelvis in
- 622 women with suspected endometriosis, including terms, definitions and
- 623 measurements: a consensus opinion from the International Deep
- 624 Endometriosis Analysis (IDEA) group. *Ultrasound Obstet Gynecol*
- 625 2016;48:318-32.

- 626 47. Vercellini P, Consonni D, Dridi D, Bracco B, Frattaruolo MP, Somigliana E.
627 Uterine adenomyosis and in vitro fertilization outcome: a systematic review
628 and meta-analysis. *Hum Reprod* 2014; 29:964–77.
- 629 48. Hjort Hansen MV, Dalsgaard T, Hartwell D, Skovlund CW, Lidegaard O.
630 Reproductive prognosis in endometriosis. A national cohort study. *Acta
631 Obstet Gynecol Scand* 2014;93:483–9.
- 632 49. Barcelos ID, Vieira RC, Ferreira EM, Martins WP, Ferriani RA, Navarro PA.
633 Comparative analysis of the spindle and chromosome configurations of in
634 vitro-matured oocytes from patients with endometriosis and from control
635 subjects: a pilot study. *Fertil Steril* 2009; 92:1749-52.
- 636 50. Mansour G, Sharma RK, Agarwal A, Falcone T. Endometriosis-induced
637 alterations in mouse metaphase II oocyte microtubules and chromosomal
638 alignment: a possible cause of infertility. *Fertil Steril*. 2010;94:1894-9.
- 639 51. De Broi MG, Malvezzi H, Paz CC, Ferriani RA, Navarro PA. Follicular fluid
640 from infertile women with mild endometriosis may compromise the meiotic
641 spindles of bovine metaphase II oocytes. *Hum Reprod* 2014; 29:315-23.
- 642 52. Practice Committee of the American Society for Reproductive Medicine.
643 Progesterone supplementation during the luteal phase and in early
644 pregnancy in the treatment of infertility: an educational bulletin. *Fertil Steril*
645 2008;89:789-92.
- 646 53. Jones CJ, Inuwa IM, Nardo LG, Litta P, Fazleabas AT. Eutopic
647 endometrium from women with endometriosis shows altered ultrastructure
648 and glycosylation compared to that from healthy controls—a pilot
649 observational study. *Reprod Sci* 2009;16:559–72.
- 650 54. Sharpe-Timms KL, Nabli H, Zimmer RL, Birt JA, Davis JW. Inflammatory
651 cytokines differentially up-regulate human endometrial haptoglobin
652 production in women with endometriosis. *Hum Reprod* 2010;25:1241–50.
- 653 55. Miller DL, Jones CJ, Aplin JD, Nardo LG. Altered glycosylation in peri-
654 implantation phase endometrium in women with stages III and IV endome-
655 triosis. *Hum Reprod* 2010;25:406–11.
- 656 56. Nirgianakis K, Bersinger NA, McKinnon B, Kostov P, Imboden S, Mueller
657 MD. Regression of the inflammatory microenvironment of the peritoneal
658 cavity in women with endometriosis by GnRHa treatment. *Eur J Obstet
659 Gynecol Reprod Biol* 2013;170:550-4.
- 660 57. Nirgianakis K, Grandi G, McKinnon B, Bersinger N, Cagnacci A, Mueller M.
661 Dienogest mediates midkine suppression in endometriosis. *Hum Reprod*
662 2016;31:1981-6.
- 663 58. Olivares C, Ricci A, Bilotas M, Barañao RI, Meresman G. The inhibitory
664 effect of celecoxib and rosiglitazone on experimental endometriosis. *Fertil
665 Steril* 2011;96:428-33.
- 666 59. Jaques AM, Amor DJ, Baker HW, Healy DL, Ukoumunne OC, Breheny S, et
667 al. Adverse obstetric and perinatal outcomes in subfertile women conceiving
668 without assisted reproductive technologies. *Fertil Steril* 2010;94:2674-9.
- 669 60. Barbosa, M. A. P., Teixeira, D. M., Navarro, P. A. A. S., Ferriani, R. A.,
670 Nastri, C. O., Martins, W. P. Impact of endometriosis and its staging on
671 assisted reproduction outcome: systematic review and meta-analysis.
672 *Ultrasound Obstet Gynecol* 2014;44:261–78.
- 673 61. Malhotra N, Karmakar D, Tripathi V, Luthra K, Kumar S. Correlation of
674 angiogenic cytokines-leptin and IL-8 in stage, type and presentation of
675 endometriosis *Gynecol Endocrinol* 2012;28:224-7.

- 676 62. Miller JE, Ahn SH, Monsanto SP, Khalaj K, Koti M, Tayade Ch. Implications
677 of immune dysfunction on endometriosis associated infertility. *Oncotarget*
678 2017;8:7138-47.
679
- 680 63. Yuge A, Nasu K, Matsumoto H, Nishida M, Narahara H. Collagen gel
681 contractility is enhanced in human endometriotic stromal cells: a possible
682 mechanism underlying the pathogenesis of endometriosis-associated
683 fibrosis. *Hum Reprod* 2007;22:938-9.
- 684 64. Nakahara K, Saito H, Saito T, Ito M, Ohta N, Sakai N, et al. Incidence of
685 apoptotic bodies in membrana granulosa of the patients participating in
686 an in vitro fertilization program. *Fertil Steril* 1997;67:302–8.
- 687 65. Lachapelle MH, Hemmings R, Roy DC, Falcone T, Miron P. Flow cytometric
688 evaluation of leukocyte subpopulations in the follicular fluids of infertile
689 patients.
690 *Fertil Steril* 1996;65:1135–40.
- 691 66. Juneau C, Kraus E, Werner M, Franasiak J, Morin S, Patounakis G et al.
692 Patients with endometriosis have aneuploidy rates equivalent to their age-
693 matched peers in the in vitro fertilization population. *Fertil Steril*. 2017 [Epub
694 ahead of print
- 695 67. Garrido N, Navarro J, García-Velasco J, Remoh J, Pellicer A, Simon C. The
696 endometrium versus embryonic quality in endometriosis-related infertility.
697 *Hum Reprod Update* 2002;8:95–103.
- 698 68. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric
699 and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a
700 systematic review and meta-analysis. *Hum Reprod Update* 2012; 18:485-
701 503.
- 702 69. Schieve LA, Tatham L, Peterson HB, Toner J, Jeng G. Spontaneous
703 abortion among pregnancies conceived using assisted reproductive
704 technology in the United States. *Obstet Gynecol* 2003; 101:959-67.
- 705 70. Pittaway DE, Vernon C, Fayez JA. Spontaneous abortions in women with
706 endometriosis. *Fertil Steril* 1988;50:711-5.
707